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FULL-LENGTH RESEARCH ARTICLE

Effect of Allelic Variation in Triticin on Bread- and Chapati-Making Qualities of Wheat (*Triticum aestivum*)

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Abstract Triticin, a legumin-like storage protein of wheat endosperm, was discovered nearly three decades ago but so far there is no report on its effect on the processing quality of wheat that is thought to be determined primarily by prolamins, its major seed storage proteins. To investigate the effect of different classes of seed proteins on wheat quality using a genetic reconstitution approach, we produced 31 near-isogenic lines (NILs) with different alleles of triticin, high molecular weight glutenin subunits (HMW-GS), low molecular weight glutenin subunits (LMW-GS), gliadins and albumins in a common genetic background of wheat variety HD2329 and analysed different quality parameters over a period of 4 years. The NILs did not differ in their flour protein content, but showed significant differences in SDS-sedimentation volume, Farinograph dough stability, bread loaf volume and chapati quality score. Main focus was on triticin for which two NILs with alleles *Tri-A1a* and *Tri-D1a* derived from a high-quality Indian wheat variety K68 were analysed. Positive effects of these triticin alleles on dough physical properties, bread loaf volume and chapatti quality score were quite large, comparable to the widely known effect of HMW-GS 5 + 10. Specific alleles of HMW-GS, *Glu-A1a* (subunit 1), *Glu-B1b* (subunits 7 + 8), *Glu-B1i* (subunits 17 + 18) and *Glu-D1d* (subunits 5 + 10) showed strong positive effects, whereas null allele *Glu-A1c* showed negative effect on the quality of recipient variety HD2329. Similarly, different alleles of LMW-GS showed varying effects with *Glu-A3d, Glu-A3e* and *Glu-D5a* showing positive effects, *Glu-A3c* showing negative effect and *Glu-A3a* showing no significant effect. Gliadin alleles generally showed negative effects, whereas albumins showed no significant effect. While results with glutenin and gliadin alleles were as expected, we show here for the first time a significant effect of triticin on the wheat flour quality, suggesting that end-use quality of wheat varieties can be improved by combining specific alleles of triticin.

Keywords Bread-making quality · Glutenin subunits · Near-isogenic lines · Triticin · Wheat

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Introduction

The end-use quality of wheat grain is determined by its protein, starch and lipids constituents of whom gluten proteins play a pivotal role. At the turn of the twentieth century, wheat seed proteins were grouped based on their solubility properties into four classes namely albumin, globulin, gliadin and glutenin [16]. While albumin and globulin are minor proteins of the wheat endosperm and are not known to greatly influence its end-use quality; gliadin and glutenin, the two major components of gluten, are the key determinants of wheat flour quality for making bread, biscuit, noodle and other products [24, 26, 27].

Fractionation and reconstitution studies with wheat flour have shown that polymeric glutenin is responsible for the strength or elasticity of wheat flour dough, whereas monomeric gliadin is responsible for its viscosity [14, 15]. Native glutenin fraction is a complex polymer mainly composed of high molecular weight (HMW) and low molecular weight (LMW) subunits whose allelic differences are known to affect the bread-making quality of wheat [5, 11, 19]. The native glutenin fraction also contains small proportion of globulins and albumin but their role in determining wheat end-use quality is not known [15]. Apart from the role of individual glutenin subunits, studies on native proteins without reduction of their disulphide bonds have shown that dough strength and bread-making quality are positively correlated with the proportion and molecular size distribution of polymeric proteins in the total flour protein [5, 9, 33, 34]. Large glutenin polymers are formed by inter-polypeptide disulphide bonds, which give wheat flour dough its unique visco-elastic properties. The HMW subunits of glutenin are encoded by *Glu-A1*, *Glu-B1* and *Glu-D1* genes located on the long arm of wheat chromosomes 1A, 1B, and 1D, respectively [11, 18]. The *Glu-D1* locus is shown to have the single largest effect on bread-making quality, followed by *Glu-B1* and *Glu-A1* loci [11]. The LMW subunits of glutenin are coded by *Glu-A3*, *Glu-B3* and *Glu-D3* genes located on the short arm of chromosomes 1A, 1B and 1D, respectively, tightly linked to the *Gli-1* loci coding for gliadins [31]. The LMW subunits have been quite difficult to study by electrophoresis due to their overlapping size with gliadin polypeptides but after development of a simplified SDS-PAGE procedure, it was shown that allelic differences at *Glu-A3*, *Glu-B3* and *Glu-D3* loci coding for LMW glutenin subunits are equally important in determining the dough properties and bread-making quality [5, 8, 13, 35].

Gliadins are monomeric proteins and when fractionated by acidic starch or polyacrylamide gel electrophoresis (APAGE), they separate into four groups, namely α, β, γ and ω gliadins [40]. Gliadin synthesis is controlled by several related genes of a limited number of multi-gene families located on the short arm of group 1 and 6 chromosomes [41]. Combined studies of HMW glutenin subunits and gliadin composition in different wheat cultivars and progenies have revealed their relative contribution to dough properties [14, 15]. It has been suggested that the effect of gliadins on dough quality should be attributed to their tight genetic linkage with LMW glutenin subunits genes [17]. Purified gliadin is known for its negative effect on dough strength and bread-making properties, and therefore, positive effect of specific *Gli-1*/*Glu-3* complex on dough resistance and extensibility is most likely due to the genetically linked *Glu-3* alleles due to their polymerization properties [5, 8, 14, 15].

Triticin is a minor seed storage globulin of the wheat endosperm first identified by Singh and Shepherd [28] and subsequently characterized in much detail at the genetic, biochemical, physiological and molecular level. [3, 25, 28, 29, 31, 32, 36]. Triticin genes are located on the short arm of wheat chromosomes 1A and 1D, near the centromere far away from the major *Gli-1/Glu-3* loci [28, 31]. Triticin is synthesized specifically during the wheat seed development and is deposited in the electron dense inclusion bodies within the main storage protein bodies of the endosperm [3, 29]. Unlike the glutenin and gliadin which are prolamin type proteins (rich in proline and glutamine amino acids), triticin shows homology to the 11-12S legumin-like storage globulins of leguminous species [32, 36]. However, the role of triticin in determining wheat dough properties and bread-making quality has not yet been investigated. The aim of this study was to develop a set of near-isogenic lines (NILs) with different alleles of triticin, HMW and LMW glutenin subunits, gliadins and albumins in a common genetic background of Indian bread wheat variety HD2329 and analyse their quality parameters. The variety HD2329 was chosen for its medium bread-making quality so that both positive and negative effects of individual alleles can be observed easily.

Materials and Methods

Plant Material and Field Experiments

A set of 31 NILs with different alleles of HMW-GS, LMW-GS, gliadin, triticin and albumin were used (Table 1). NILs were produced by crossing a highly adaptable bread wheat variety HD2329 with donor wheat varieties having different seed storage protein alleles followed by three backcrosses coupled with phenotypic and AFLP marker-based background selection and protein electrophoresis-based foreground selection [22, 23]. Segregating populations for triticin alleles were developed by crossing triticin NILs with the recipient variety HD2329. Two such segregating populations were developed, one each for the *Tri-A1* and *Tri-D1* loci. Homozygous lines for the two triticin alleles were selected from the F_2 progeny by SDS-PAGE on endosperm half of the seed, while embryo half was grown to obtain F_3 seeds for quality analysis. Seeds of all the NILs were multiplied in the experimental fields of IARI, New Delhi, using a completely randomized block design in two replicates with a plot size of 100 plants each sown in a 6 × 6 grid design of 3 m × 3 m during the *Rabi* seasons of 2007–2010. The field managements were carried out according to standard practices for wheat, and mature grains were harvested for analysis of quality traits. F_3 families of triticin segregating lines were grown in a net

Table 1 Near-isogenic lines (NILs) with different seed storage protein alleles in the background of wheat variety HD2329

S. no.	NIL no.	Donor locus allele	Donor parent	HD2329 allele
1	TRI-1	*Tri-A1a*	K-68	*b*
2	TRI-2	*Tri-D1a*	K-68	*b*
3	HMW-1	*Glu-A1a (1)*	UP2121	*b* (2*)
4	HMW-2	*Glu-A1c* (*Null*)	CS-1BL	*b* (2*)
5	HMW-3	*Glu-B1b* (*7 + 8*)	CS1A-1B	*b* (7 + 9)
6	HMW-4	*Glu-B1b** (*7* + 8*)	UP1109	*b* (7 + 9)
7	HMW-5	*Glu-B1i (17 + 18)*	Kalyansona	*b* (7 + 9)
8	HMW-6	*Glu-D1d (5 + 10)*	K-68	a (2 + 12)
9	LMW-1	*Glu-A3c*	Kalyansona	*b*
10	LMW-2	*Glu-A3e*	UP115	*b*
11	LMW-3	*Glu-A3d-1*	UP319	*b*
12	LMW-4	*Glu-A3d-2*	UP1109	*b*
13	LMW-5	*Glu-A3a*	CS-1BL	*b*
14	LMW-6	*Glu-D5a*	CS1A-1B	*b*
15	LMW-7	*Glu-B3ks*	Kalyansona	*hd*
16	GLI-1	*ω-Gli-B1d*	Kalyansona	*hd*
17	GLI-2	*ω-Gli-D1b*	WH147	*hd*
18	GLI-3	*ω-Gli-B1a*	CS1A-1B	*hd*
19	GLI-4	*ω-Gli-B1c*	UP1109	*hd*
20	GLI-5	*ω-Gli-A1g*	WH147	*hd*
21	GLI-6	*ω-Gli-A1h*	UP115	*hd*
22	GLI-7	*ω-Gli-B1b*	UP2121	*hd*
23	GLI-8	*γ- Gli-ma*	UP1109	*hd*
24	GLI-9	*γ- Gli-mb*	WH147	*hd*
25	GLI-10	*γ- Gli-mc*	UP319	*hd*
26	GLI-11	*γ- Gli-md*	UP115	*hd*
27	GLI-12	*γ- Gli-me*	Sunkota B	*hd*
28	ALB-1	*Alb-mb*	UP301	*hd*
29	ALB-2	*Alb-mc*	UP115	*hd*
30	ALB-3	*Alb-mf*	UP1109	*hd*
31	ALB-4	*Alb-mg*	K-68	*hd*

house to obtain enough F4 seeds for the quality analysis using SDS sedimentation and extensigraph tests.

Protein Extraction and SDS-PAGE

Sequential extraction of seed albumin, gliadin and glutenin were done from crushed endosperm half of single seed (~15 mg) or 20 mg of four samples in 1.5 ml Eppendorf tubes. First, albumin was extracted in 200 µl of RO water (18 Ohm) at 25 °C for 30 min, centrifuged at 15000×*g* for 10 min, and then 100 µl of the supernatant was mixed with equal volume of 2× sample buffer [2 % SDS, 20 % (w/v) glycerol, 82.5 mM tris-base, 0.2 % bromophenol blue, pH 8.0] containing 1 % (v/v) dithiothreitol. The residue was washed with 0.5 ml of RO water and then extracted with 200 ul of 50 % (v/v) propan-2-ol, centrifuged at 15,000×*g* for 10 min as reported earlier [22, 23]. Except for albumins which were extracted at room temperature, all other seed storage proteins were extracted by incubation at 60 °C for 10–15 min just before loading in the gel. Glutenin (HMW-GS and LMW-GS) extraction and separation were done according to Singh et al. [35]. Albumins (30 µl), glutenins (25 µl) and gliadins (20 µl) extracts were separated in a 10 % polyacrylamide gels with 1.5 % cross-linking. Electrophoresis was performed in 1.5 mm thick slab gels of 20 × 20 cm dimension using Hoefer SE600 electrophoresis system at a constant current of 40 mA/gel for 2.5 h. Triticin was extracted from single seeds in 1 M NaCl and precipitated with acetic acid as described in Singh et al. [30]. The triticin gels were run for a longer period of 3.5 h for better resolution of high molecular weight triplet protein bands. After electrophoresis, gels

were stained in coomassie brilliant blue solution [6 % (w/v) trichloroacetic acid, 18 % methanol, 6 % glacial acetic acid and 0.025 % coomassie brilliant blue R250] overnight and destained in 3 % NaCl solution as described by Sreeramulu and Singh [38].

Measurement of Quality Traits

Grain Protein Content and Kernel Characteristics

Grain protein content (GPC) was measured by near infrared reflectance spectrometry (NIRS) from grain samples according to the AACC method [1]. White flour protein content and moisture level were also measured by NIRS and used together with grain hardness index for calculating the amount of water required for Farinograph test [Brabender 1965]. Grain hardness (GH), moisture content (MC), grain diameter (GD) and thousand kernel weight (TKW) were measured on 300 kernels for each sample using Perten Single Kernel Characterization System (SKCS) 4100 system, following manufacturer's protocol (Perten Instruments North America Inc., Springfield, IL).

SDS-Sedimentation Volume Test

Flour samples were evaluated for bread-making quality using SDS-sedimentation volume (SDS-SV) test [2]. In this method, the volume of material which sediments after mixing flour with a solution of SDS and lactic acid is measured. Milling of grains was performed in a Brabender Junior mill to obtain flour extraction rate of 60 %.

Farinograph Test

Farinograph curves (C.W. Brabender Instruments, Inc., South Hackensack, NJ, USA) were generated according to the AACC method [1]. The 50 g mixing bowl was used in conjunction with the standard operating speed of 63 rpm. The curves were read manually, and different parameters were recorded, including Farinograph water absorption (FAB, 14.0 % moisture basis), the amount of water required to centre the curve on the 500 BU line; dough stability (STA), the difference in time from when the top of the curve first reaches the 500 BU line (arrival time, AT) to when it first leaves the 500 BU line (departure time, DT); mixing tolerance index (MTI), the drop in the curve 5 min after peak development, measured in BU units; dough development time (DDT), the time required to reach peak dough development; and time to breakdown (TTB), the time from the start of mixing to the time at which the consistency decreases 30 BU from the peak.

Dough Resistance and Extensibility

The segregating lines of triticin were analysed using texture analyser for extensigraph properties. This was done by TA.XT*plus* Texture Analyser from Stable Micro Systems using Kieffer extensibility rig. It uses the same principle as Brabender Extensograph, except that the sample is stretched upwards and the dough requirement is low. It provides information about dough resistance to stretching and extensibility by measuring the force to pull a hook through a cylindrically shaped piece of dough [43].

Bread Loaf Volume

Baking performance was evaluated by doing an optimized straight-dough bake test (Approved Method 10-10B, AACC 1995) using 100 g of flour (14 % moisture basis). Optimum bake water absorption (%WA) and mixing time (min) were those resulting in dough with optimal handling characteristics as judged by bakers. Loaf volume (cm^3) was determined by rapeseed displacement method on fresh loaves.

Chapati-Making Quality

Chapatis were prepared from whole-wheat flour according to the method developed by Haridas Rao et al. [10]. Flour (200 g, 14.0 % moisture basis) and water as determined from a 500 B.U. Farinograph trace were mixed in a Hobart dough kneader (HL 120 Hz 50/60) for 5 min. The dough was rested for 10 min before being cut into four equal sections of 40 g each. A section of the dough was then placed on a rolling board with a thickness guide of 1.5 mm. The dough was rolled in one direction, inverted, rotated at 90° and rerolled. The sheeted dough was cut with a circular die to get a 12-cm diameter uniform chapati (Fig. 1a). The raw chapati was placed on a preheated griddle at 215 °C. The chapati was cooked for 70 s on one side, flipped, and then cooked for 85 s on the second side. The cooked chapati was quickly transferred (<10 s) to an adjacent heater and allowed to puff for 20 s before removal and cooling at room temperature for 10 min.

Puffing height was recorded by a scale with sliding bar (Fig. 1b). Puffing height of chapati between 0 and 5 cm chapati was given 5 points. Chapati was also evaluated by a trained panel of four judges and scored (0–10) subjectively for the following quality parameters; appearance, tearing strength, pliability, aroma and eating quality (0–15). After taking one set of observations, chapati was placed in a re-sealable plastic bag and stored for 4 h before next round of evaluation. Again after 4 h chapattis were evaluated for tearing strength and pliability (score 0–10). The higher the score, the better the quality of chapatti. Assessments were

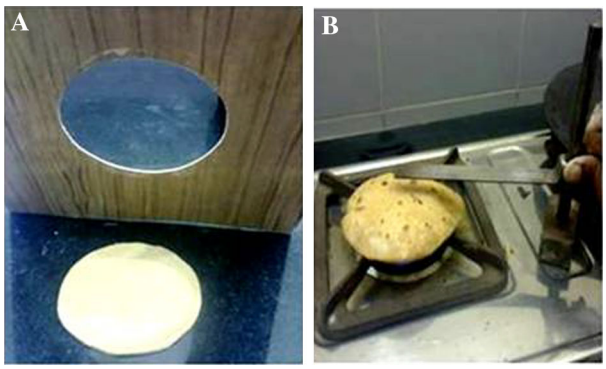

Fig. 1 Simple equipment used for chapati making and puffing. **a** cutting of uniform size chapati using a plastic manifold with round hole, **b** measurement of chapati puffing height

made in duplicate, and scores of all the panellists were averaged.

Statistical Analysis

Analysis of variance (ANOVA) was done using SPSS software package ver. 16. The seed samples of all 4 years were taken for the analysis of SDS sedimentation and protein content, while only two-year seeds were used for Farinograph and baking quality tests. *T* test was performed for assessing the significance of differences among the means for the NILs and triticin segregating lines at 0.5 % *P* level of significance.

Results

Characterization of Seed Protein NILs

Total thirty-one NILs were developed with different seed storage protein alleles in the common genetic background of bread wheat variety HD2329. Different seed protein genes, the allele for which individual NIL differed from the recipient variety HD2329, donor variety and corresponding allele in the recipient variety are shown in Table 1. Genes and alleles for which no recognized symbols are available, e.g. albumin polypeptides, were assigned new temporary symbols [23]. Field observations over 4 years showed that the NILs were quite similar in appearance and yield performance to the recipient variety HD2329, except for white glume colour in some of the NILs for *Gli-B1/Glu-B3* loci, namely [GLI-1,3,4,7 and LMW-7], and the rest of NILs were brown in glume colour like recipient variety HD2329. This was due to a tight genetic linkage between gene for red glume colour and *Gli-B1/Glu-B3* locus on the short arm of chromosome 1B [12].

The NILs were characterized for their complete seed protein profile by SDS-PAGE to check for similarity of non-target protein loci with the recipient parent HD2329. There were two NILs with different alleles of triticin, namely TRI-1 and TRI-2, corresponding to triticin alleles *Tri-A1a* and *Tri-D1a*, respectively (Fig. 2). The recipient variety HD2329 had *Tri-A1b* and *Tri-D1b* alleles at these loci resulting in a narrow triplet band compared to NIL TRI-1 which had a faster moving Tri-A1 band and NIL-2 which had a slower moving Tri-D1 band resulting in wider triplet bands in the two NILs. The intensity of triticin bands was also consistently darker in the two NILs as compared to HD2329. Both the triticin NILs showed identical electrophoretic profiles for HMW-GS, LMW-GS, gliadin and albumin fractions.

There were six HMW-GS NILs, two for *Glu-A1* locus (*Glu-A1a* and *Glu-A1c* with subunit 1 and null, respectively), three for *Glu-B1* locus (*Glu-B1b, Glu-B1b** and *Glu-B1i* with subunit 7 + 8, 7* + 8 and 17 + 18, respectively) and one for the *Glu-D1* locus (*Glu-D1d* with subunit 5 + 10). SDS-PAGE analysis established that the

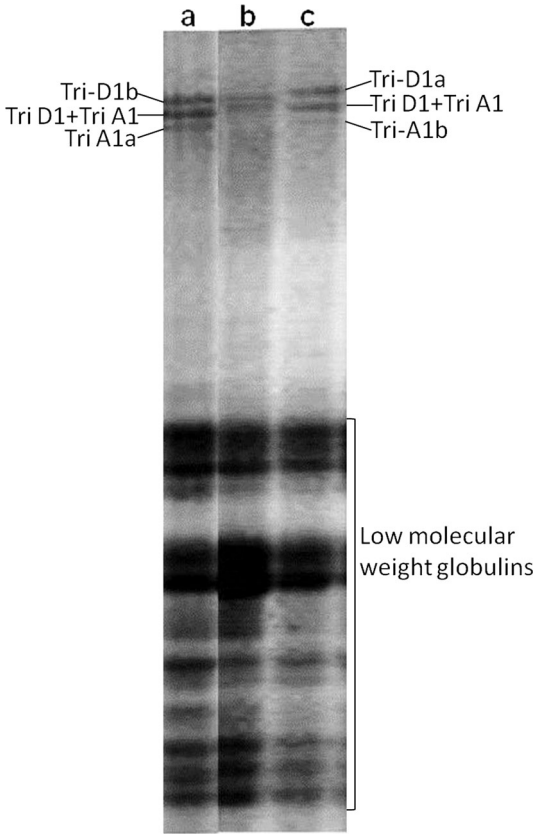

Fig. 2 SDS-PAGE patterns of NILs for triticin seed storage globulins. *a* NIL Tri-A1a, *b* Recipient parent HD2329, *c* NIL Tri-D1a. Slowest moving dark band in the triplet is pure Tri-D1, fastest moving faint band is pure Tri-A1, whereas middle dark band is a heteromer of Tri-D1 and Tri-A1 subunits

HMW-GS NILs differed for the targeted HMW subunit only, and their profiles for other HMW subunits, LMW subunits, gliadin, albumin and triticin proteins were identical to the recurrent parent HD2329 (Fig. 3). There were seven NILs for LMW glutenin subunits of which five were for *GluA3* locus (*Glu-A3a, Glu-A3c*, two NILs with *Glu-A3d* from separate donors and *Glu-A3e*), one each for *Glu-D5* (*Glu-D5a*) and an uncharacterized locus "*Glu-B3ks*" from donor variety Kalyansona (Fig. 4a). The HMW glutenin subunits, albumins and triticin profiles of the LMW-GS NILs were identical to the recipient parent HD2329, but there were differences in their gliadin profiles due to tight linkage between genes for LMW glutenin subunits and gliadins (Fig. 4b). Only two of the seven LMW-GS NILs namely LMW-2 and LMW-6 showed gliadin patterns identical to HD2329, and the remaining five LMW-GS NILs showed differences in the ω- and γ-gliadin regions as marked in Fig. 4b. Interestingly, NILs LMW-3 and LMW-4 both have the same LMW *Glu-A3d* allele but they differ for gliadin patterns. LMW-3 NIL has different ω-gliadin pattern which can be due to some insufficient backcrossing or rare recombination between *Glu-A3* and *GliA1* loci while LMW-4 NIL gliadin pattern is similar to HD2329. There were 12 NILs with different gliadin alleles; seven of these (Gli-1 to Gli-7) differed in the ω-gliadin region, while remaining five (Gli-8 to Gli-12) differed in the γ-gliadin region (Fig. 5a). SDS-PAGE analysis showed that similar to the LMW-GS NILs, gliadin NILs showed differences in their LMW-GS profiles due to tight genetic linkage between the two loci (Fig. 5b). The LMW-GS are divided into two groups, B and C subunits, based on their size distribution. Variation in the LMW-GS of these NILs was mainly in the slower moving B group of subunits, variation in the C group of subunits was limited to absence in the γ–gliadin NILs of one of the three subunits of

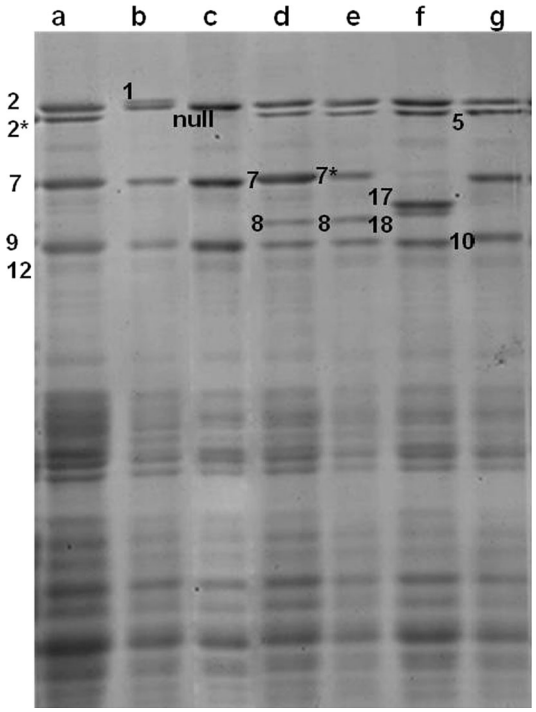

Fig. 3 SDS-PAGE patterns of HMW glutenin subunits (HMW-GS) NILs. *a* Recipient parent HD2329, *b* NIL GluA1a, *c* NIL GluA1c, *d* NIL Glu-B1b, *e* NIL Glu-B1b*, *f* NIL Glu-B1i, *g* NIL Glu-D1d. Relevant HMW-GS subunits are marked

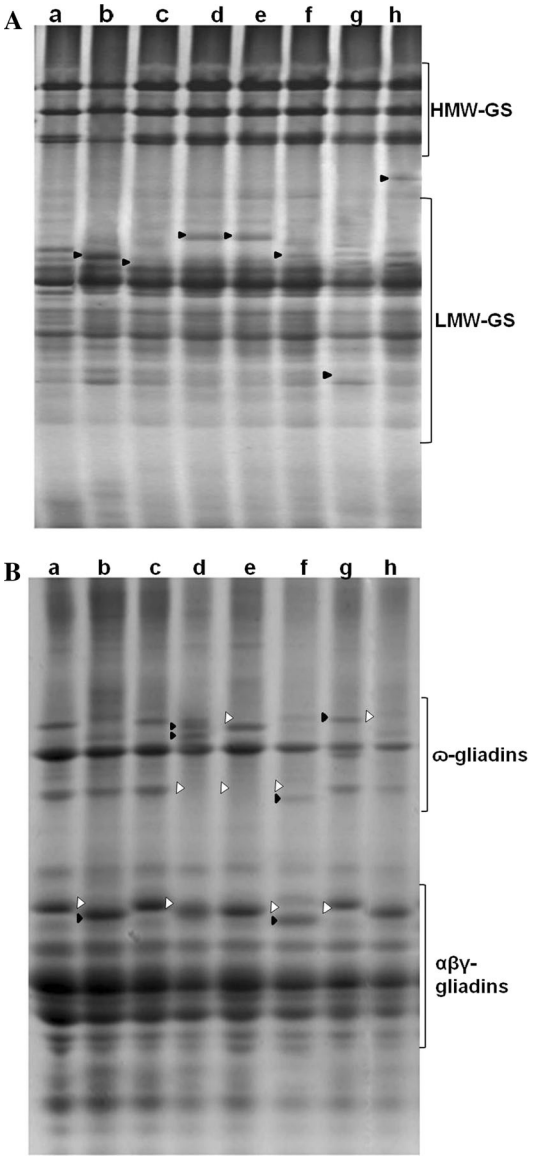

Fig. 4 SDS-PAGE pattern of NILs for LMW glutenin subunits (LMW-GS). **a** Separation of LMW-GS alleles (marked with *right pointing triangle*, missing bands marked with *open triangles*). **b** Separation of gliadins from the LMW-GS NILs. *a* Recipient parent HD2329, *b* NIL Glu-A3c, *c* NIL Glu-A3e, *d* NIL Glu-A3d-1, *e* NIL Glu-A3d-2, *f* NIL Glu-A3a, *g* NIL Glu-D5a, *h* NILGlu-B3ks. Differences in gliadin patterns of LMW-GS NILs are due to tight linkage between the two loci

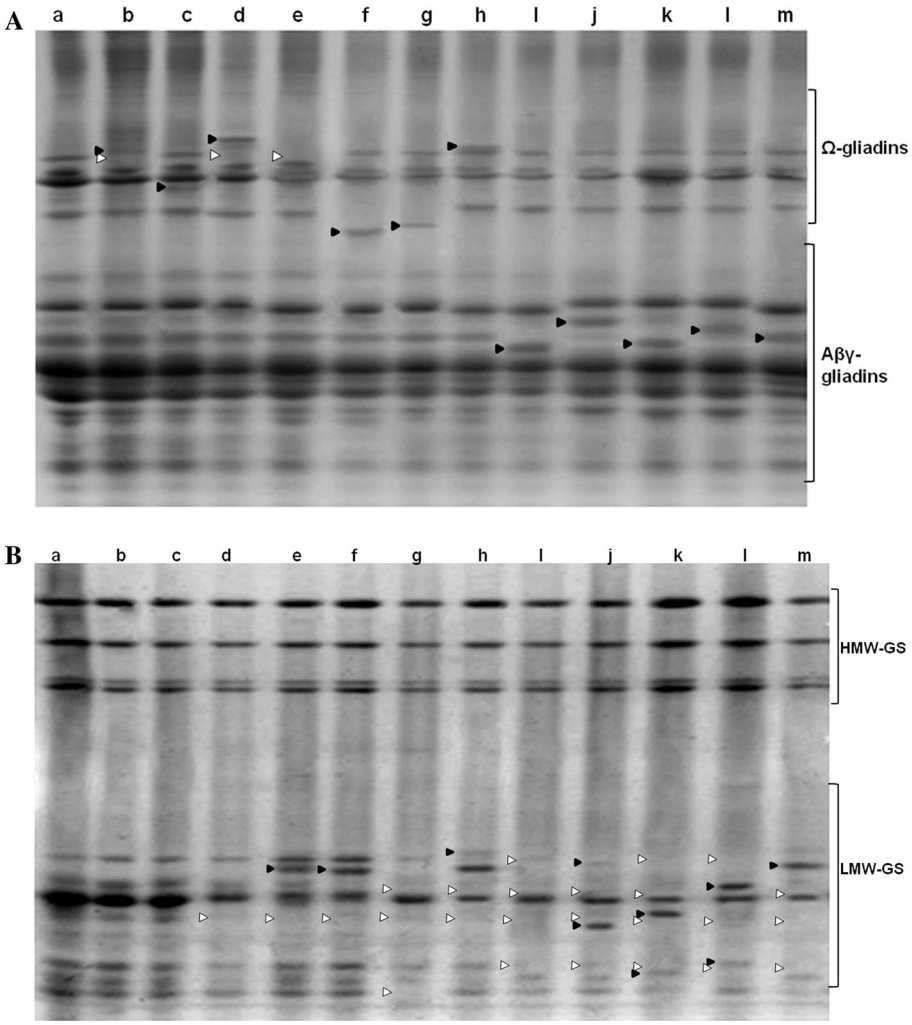

Fig. 5 SDS-PAGE pattern of NILs for Gliadins. **a** Separation of gliadins alleles (marked with *right pointing triangle*, missing bands marked with *open triangles*). **b** Separation of LMW glutenin subunits from the gliadin NILs. *a* Recipient parent HD2329, *b* NIL Gli-B1d, *c* NIL Gli-D1b, *d* NIL Gli-B1a, *e* NIL Gli-B1c, *f* NIL Gli-A1g, *g* NIL Gli-A1h, *h* NIL Gli-B1b, *i* NIL γ-gli ma, *j* NIL γ-gli mb, *k* NIL γ-gli mc, *l* NILγ-gli md, *m* NILγ-gli me. Differences in LMW glutenin subunit patterns of gliadin NILs are due to tight linkage between the two loci

HD2329. HMW-GS, albumin and triticin profiles of the gliadin NILs were identical to the recipient variety HD2329. There were four NILs with different albumin alleles for which temporary new symbols *Alb-mc, Alb-mf, Alb-mb* and *Alb-mg* were assigned as there were no gene symbols available for these in the literature (Fig. 6).

Effect of Allelic Variation in Seed Proteins on Quality

The thirty-one NILs were analysed for a range of grain quality parameters considered important for the end-use products namely bread, biscuit and chapati. A single kernel characterization system (SKCS) was used for the analysis of grain hardness, TKW and grain moisture content but these traits did not show significant variation among the NILs, except for TKW which varied significantly between 33.5 and 39.5 g, which is important for flour yield during milling of the wheat grains (Table 2). Further there was no significant difference for grain protein content among the NILs and recipient variety HD2329 (Table 2). This shows that different alleles of seed storage proteins had no relationship with the above quality parameters. However, there were significant differences among NILs for SDS-sedimentation volume, Farinograph dough development time, dough stability and bread loaf volume, showing that these parameters were affected by the seed storage protein allelic composition. Thirty-one NILs analysed in this study represented all four classes of seed proteins. The major storage proteins glutenin and gliadin showed significant positive or negative effect on the dough and bread-making quality of the base wheat variety HD2329, whereas albumin NILs showed no significant effect as also described in the published literature [21]. The most important novel finding of our study was significant positive effect of triticin on wheat quality parameters. Effect of allelic variation in seed protein on different wheat quality parameters are described below.

SDS-Sedimentation Volume

Sodium dodecyl sulphate-sedimentation volume (SDS-SV) measures degree of sedimentation of wheat flour suspended in a lactic acid-SDS medium during a standard time of

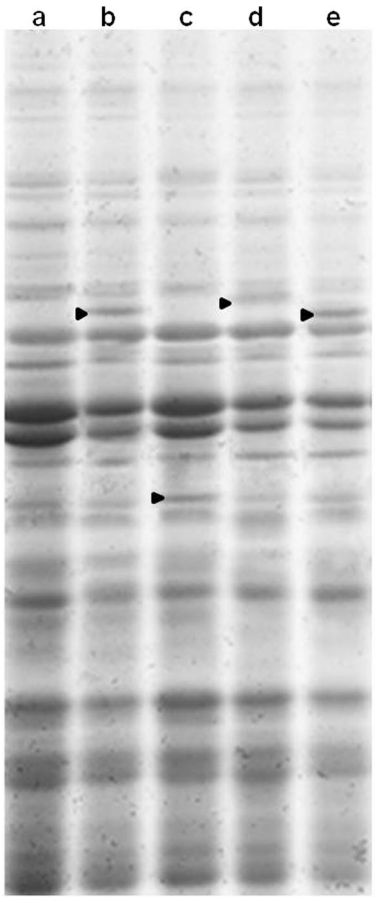

Fig. 6 SDS-PAGE pattern of NILs for seed albumins. Relevant albumin proteins are marked with *right pointing triangle*. *a* recipient parent HD2329, *b* NIL Alb-mc, *c* NIL Alb-mf, *d* NIL Alb-mg, *e* NIL Alb-mh

settling [2]. The SDS-SV value depends on the protein quality and provides an indication of wheat gluten strength. Triticin NILs, with alleles *Tri-A1a* and *Tri-D1a*, showed highly significant positive effect on SDS-SV, which was comparable to the effect of HMW glutenin subunits 5 + 10 known for their strong positive impact on bread-making quality [17]. Effect of *Tri-D1a* was comparatively more pronounced than *Tri-A1a* (Table 2). The HMW-GS NILs with alleles *Glu-A1a* (subunit *1*), *Glu-B1b* (subunit *7 + 8*), *Glu-B1b** (subunit *7* + 8*), *Glu-B1i* (subunit *17 + 18*) and *Glu-D1d* (subunit *5 + 10*) showed significant positive effect on SDS-SE of HD2329, while *Glu-A1c* (Null allele) showed significant negative effect in 4 years of evaluation (Table 2). All the HMW-GS alleles were positively behaving towards SDS-SV, and only *Glu-A1c* (Null allele) was behaving negatively. There were seven NILs for LMW-GS, five of which represented different alleles of *Glu-A3* locus. LMW NILs for allele *Glu-A3c* were showing significantly negative effect from HD2329 and that of *Glu-A3a* was showing no effect, and the rest of the LMW NILs (*Glu-A3e, Glu-A3d, Glu-D5a and Glu-B3ks*) were showing positive effect over recurrent parent for SDS-SV test. Two separate NILs with *Glu-A3d* showed strong positive effect over recurrent parent HD2329 but effect of NIL Glu-A3d-1 was lower than Glu-A3d-2 (Table 2). This could be due to difference in the linked gliadin polypeptides as Glu-A3d-1 has multiple ω-gliadin bands which have negative effect of dough strength. All the twelve gliadin NILs showed either negative or no significant effect on the SDS-SV of recipient variety HD2329. The albumin NILs showed no significant effect on SDS-SV.

Farinograph Physical Dough Properties

Similar to the effect on SDS-SV both the triticin NILs, Tri-A1a and Tri-D1a, showed highly significant positive effect on dough stability but no effect on farinograph dough development time (Table 2). HMW glutenin subunit NILs with alleles *Glu-A1a (1), Glu-B1b (7 + 8), Glu-B1b* (7* + 8), Glu-B1i (17 + 18)* and *Glu-D1d (5 + 10)* showed significant positive effects on Farinograph dough stability over HD2329, while *Glu-A1c* (null allele) showed significant negative effect. LMW-GS NILs Glu-A3c showed significant negative effect on Farinograph dough stability, while Glu-A3a showed no effect rest all the LMW NILs (Glu-A3e, Glu-A3d, Glu-D5a and Glu-B3ks) showed positive effect over recurrent parent for Farinograph dough stability test. Farinograph dough development time showed no significant difference over HD2329 except for allele *Glu-D5a* which has a low dough development time. All the gliadin NILs, except γ-Gli-me and ω-Gli-B1b showed significant negative effect, while albumin NILs either showed significantly negative (Alb-mf) or no significant effect (Alb-ma, Alb-mb and -Alb-mg) on dough stability (Table 2). All the gliadin alleles showed no significant effect except ω-Gli-B1c, ω-Gli-A1g and γ-Gli-md which behaved negatively for dough development time. All the albumins also showed low dough development time.

Bread Loaf Volume

As expected from the data on SDS-SV and Farinograph physical dough properties, triticin alleles *Tri-A1a* and *Tri-D1a* showed highly significant positive impact on the loaf volume of wheat variety HD2329 that was consistent in 2 years of testing. The effect of triticin alleles *Tri-A1a* and *Tri-D1a* coming from a traditional high-quality wheat variety K68 was as high as the effect of well-known HMW glutenin subunits 5 + 10. The bread loaf volume was 620 cc for *Glu-D1d*, whereas it was 630 cc and 610 cc, for *Tri-D1a* and *Tri-A1a*, respectively as compared to 550 cc for HD2329. (Table 2; Fig. 7). HMW-GS (NILs Glu-A1a, Glu-B1b, Glu-B1b*, Glu-B1i and Glu-D1d) showed significant positive effect, while Glu-A1c showed significant

negative effect on the loaf volume over HD2329 (Table 2). Among the LMW-GS NILs, only Glu-A3d-1, Glu-A3d-2 and Glu-D5a showed significant positive effect on loaf volume, and the remaining LMW-GS NILs (Glu-A3c, Glu-A3e, Glu-A3a and Glu-B3ks) showed negative or no effect on bread loaf volume over HD2329. All the gliadin NILs showed significant negative effect on loaf volume except ω-Gli-B1d and ω-Gli-B1a which had no significant effect (Table 2). Albumin NILs had no significant effect on bread loaf volume as compared to the recurrent parent HD2329 (Table 2).

Chapati Quality Score

The bulk of Indian wheat is consumed in the form of chapati, and it is realized that over the years during and post green revolution era, the chapati quality of Indian wheat varieties has declined. Thus, some of the older varieties grown in the central India, so called MP wheat, still fetch premium price in the market [42]. Similar to their effect on SDS-SV, dough physical properties and bread loaf volume, triticin alleles *Tri-A1a* and *Tri-D1a* showed significant positive impact on chapati quality score also, which was consistent in 2 years of testing. We evaluated the chapati-making quality of HMW-GS and LMW-GS NILs and found that all the HMW-GS and LMW-GS NILs generally showed positive effects on the chapati quality of HD2329, except for alleles HMW-GS *Glu-A1c* and LMW-GS *Glu-A3a, Glu-A3*e, *Glu-A3*c and *Glu-D5a* which showed no significant effect. None of the gliadin and albumin alleles showed significant effect on chapati quality score.

Validation of the Effect of Triticin Alleles in Segregating Bi-Parental Populations

For further validation of the effect of triticin alleles *TriA1a* and *Tri-D1a,* two segregating populations were developed by crossing the respective triticin NILs with recipient variety HD2329. Homozygous lines with two segregating alleles of the *Tri-A1* and *Tri-D1* genes were selected by SDS PAGE. The triticin patterns of each *Tri-A1a*, *Tri-A1b, Tri-D1a* and *Tri-D1b* homozygous line are shown in Fig. 8. *Tri-A1a* and *Tri-D1a* were obtained from K68, the donor variety of triticin NILs, whereas *Tri-D1b* and *Tri-A1b* were from recurrent parent HD2329. Twenty eight such homozygous F_3 segregating lines were multiplied in the net house, and bulk F_4 seeds were harvested for quality analysis by SDS-SV and a small-scale dough Extensigraph. Effect of *Tri-D1a* and *Tri-A1a* alleles was significantly positive on SDS-SV and dough Extensiograph force over HD2329. The overall effect of all the four triticin alleles was significant on SDS sedimentation value ($P < 0.05$) and Extensiograph force but extensibility was not affected significantly (Table 3). *Tri-D1a* and *Tri-D1b* alleles gave consistently higher Extensigraph force value than HD2329 but the effect of *Tri-D1a* was higher than *Tri-D1b.* Similarly, the effect of *Tri-A1a* was higher than *Tri-A1b* allele (Fig. 8).

Discussion

A wheat cultivar can produce good quality bread even with moderate protein content, if the protein quality is good. In fact, in many breeding programmes, consciously or not, some HMW-GS alleles, in particular *Glu-D1d* (subunits 5 + 10), were frequently used for increasing end-use quality [38]. Earlier studies have provided evidence for strong association between the presence of specific HMW-GS alleles and bread-making quality [17, 20]. Further studies have shown that allelic variations in both HMW-GS and LMW-GS are important in determining the bread-making quality of wheat flour [7]. In our study, no significant difference was found in the protein content of the thirty-one NILs with different seed storage protein alleles, so the effect on wheat quality was primarily due to protein quality i.e. amino acid sequence variation of alleles. A number of studies have been done for evaluating the effects of different HMW, LMW glutenin subunits and gliadin alleles on bread-making quality of wheat but contribution of wheat triticin has not yet been investigated. Triticin is a minor seed storage protein which accounts for only about 5 % of the total endosperm protein in wheat. It is legumin-like protein and has a lysine-rich repetitive domain in its hyper variable region which offers new opportunities to genetic engineers for increasing lysine content of wheat [36]. Our study found two alleles of triticin showing significant positive effect on bread-making quality parameters. SDS sedimentation volumes were 46 and 44 ml in *Tri-D1a* and *Tri-A1a*, respectively, which are comparable to the effect of HMW-GS *Glu-D1d* with volume of 45 ml. *Glu-D1d* has already proved to be a good contributor towards bread-making quality. Triticin allele's effect was comparable to *Glu-D1d* consistently in the 4 years trials. The effect was more pronounced on dough strength where *Tri-D1a* and *Tri-A1a* NILs showed Farinograph dough stability time of 17.5 and 16 min, respectively, compared to 16 min for *Glu-D1d* NIL and 11 min for the recipient parent HD2329. Similar positive effect was seen on bread loaf volume which was over 610 cc for the triticin NILs as compared to 550 cc for HD2329.

Alleles of HMW-GS and LMW-GS showed expected effects as described in the earlier studies. All the HMW-GS alleles, except the null allele *Glu-A1c* showed positive effect on SDS-SV and Farinograph dough stability. Earlier

Table 2 Grain and flour quality scores of thirty-one near-isogenic lines with different seed storage protein alleles in wheat variety HD2329

Protein class	NIL allele	Thousand kernel weight (g)*	Grain protein content (%)*	Grain hardness index*	SDS-SV (ml)*	Farinograph		Bread loaf volume (cc)**	Chapati quality score**
						DDT (min)**	Stability (min)**		
	HD2329	36.5 [b]	12.8	92.2	35.3[c]	4.8[a]	10.5 [c]	550 [c]	60 [c]
Triticin	Tri-D1a	36.8 [b]	12.6	91.3	46.0 [a]	4.5[a]	17.3 [a]	630 [a]	79[a]
	Tri-A1a	35.8 [b]	12.6	90.9	44.0 [a]	4.8[a]	15.5 [a]	610 [a]	79 [a]
HMW-glutenin subunits	Glu-A1a	36.2 [b]	13.3	93.4	38.8 [b]	4.3 [a]	13.4 [b]	610 [a]	80 [a]
	Glu-A1c	36.7 [b]	13.4	90.1	32.8 [d]	4.6 [a]	8.0 [d]	520 [d]	65 [c]
	Glu-B1b	39.5 [a]	13.0	93.3	40.0 [b]	5.2 [a]	13.3 [b]	570[b]	79 [a]
	Glu-B1b*	34.6 [b]	13.3	92.7	39.0 [b]	5.3 [a]	13.3 [b]	580 [b]	79 [a]
	Glu-B1i	39.4 [a]	12.8	91.2	39.5 [b]	4.5 [a]	14.8 [b]	600 [a]	79 [a]
	Glu-D1d	39.5 [a]	12.9	96.4	45.0 [a]	4.5 [a]	16.3 [a]	620 [a]	79 [a]
LMW-glutenin subunits	Glu-A3c	37.7 [a]	12.6	93.7	32.0 [d]	4.3 [a]	7.8 [d]	460 [e]	60 [c]
	Glu-A3e	36.4 [b]	13.3	89.8	38.8 [b]	4.3 [a]	12.5 [b]	540 [c]	60 [c]
	Glu-A3d-1	34.6 [b]	13.2	89.5	37.0 [b]	4.7 [a]	14.8 [b]	570 [b]	70 [b]
	Glu-A3d-2	37.5 [a]	13.5	93.9	46.0 [a]	5.8 [a]	12.8 [b]	600 [a]	70 [b]
	Glu-A3a	35.7 [b]	13.4	94.1	35.5 [c]	4.4 [a]	10.4 [c]	540 [c]	65 [c]
	Glu-D5a	33.7 [b]	13.5	89.3	39.0 [b]	3.8 [b]	12.1 [b]	580 [b]	65 [c]
	Glu-B3ks	33.8 [b]	13.0	91.1	37.0 [b]	5.5 [a]	12.2 [b]	500 [d]	69 [b]
Gliadin	ω-Gli-B1d	37.2 [a]	12.8	94.8	35.0 [c]	5.5 [a]	7.8 [d]	540 [c]	59 [c]
	ω-Gli-D1b	38.4 [a]	12.8	90.7	33.0 [d]	5.3 [a]	7.8 [d]	510 [d]	59 [c]
	ω-Gli-B1a	37.9 [a]	12.6	92.7	31.0 [d]	4.3 [a]	7.1 [d]	555 [c]	59 [c]
	ω-Gli-B1c*	37.1 [a]	12.5	88.6	30.3 [d]	3.8 [b]	4.6 [e]	450 [e]	59 [c]
	ω-Gli-A1g	37.9 [a]	13.1	93.9	31.3 [d]	3.5 [b]	6.8 [d]	410 [e]	59 [c]
	ω-Gli-A1h	34.7 [b]	13.2	94.8	34.0 [c]	4.8 [a]	8 [d]	430 [e]	59 [c]
	ω-Gli-B1b	38.7 [a]	12.8	94.2	35.5 [c]	5.3 [a]	9.5 [c]	470 [e]	59 [c]
	γ-Gli-ma	39.1 [a]	12.9	92.6	33.0 [d]	4.5 [a]	7.5 [d]	510 [d]	59 [c]
	γ-Gli-mb	38.5 [a]	13.1	88.7	32.0 [d]	4.5 [a]	7.8 [d]	450 [e]	59 [c]
	γ-Gli-mc	37.9 [a]	12.9	90.2	34.0 [c]	4.4 [a]	7.5 [d]	420 [e]	59 [c]
	γ-Gli-md	35.7 [b]	12.4	90.5	34.8 [c]	4.0 [b]	7.3 [d]	470 [e]	59 [c]
	γ-Gli-me	36.3 [b]	12.6	92.8	34.5 [c]	5.3 [a]	10.5 [c]	510 [d]	59 [c]
Albumin	Alb-mb	36.1 [b]	12.5	94.2	37.0 [b]	4.0[b]	10.5 [c]	550 [c]	60 [c]
	Alb-mc	33.5 [b]	12.6	92.5	35.0 [c]	3.5 [b]	9.6 [c]	530 [c]	60 [c]
	Alb-mf	35.9 [b]	12.4	94.7	35.0 [c]	4.0[b]	6.5 [d]	555 [c]	60 [c]
	Alb-mg	36.3 [b]	12.1	95.3	34.0 [c]	4.0[b]	10.3 [c]	555 [c]	60 [c]

Differences in mean values in the same column followed by different letter in superscript are statistically significant

NIL near-isogenic lines; *SDS-SV* sodium dodecyl sulphate-sedimentation volume

* Means of 4 years data

** Means of 2 years data

Table 3 Extensigraph and SDS-sedimentation volume scores of F_4 homozygous seeds segregating for triticin alleles from crosses between HD2329 and triticin NILs

Sr. no.	Genotype	Extensiograph	
		Resistance (cm)	SDS-SV (ml)
1.	HD2329	0.022[c]	35[b]
2.	NIL Tri-A1a	0.040[b]	44[a]
3.	NIL Tri-D1a	0.086[a]	46[a]
4.	Tri-D1a-1	0.083[a]	45[a]
5.	Tri-D1a-2	0.073[a]	44[a]
6.	Tri-D1a-3	0.087[a]	45[a]
7.	Tri-D1a-4	0.077[a]	45[a]
8.	Tri-D1a-5	0.084[a]	45[a]
9.	Tri-D1a-6	0.091[a]	44[a]
10.	Tri-D1a-7	0.083[a]	44[a]
11.	Tri-D1a-8	0.087[a]	44[a]
12.	Tri-D1a-9	0.082[a]	44[a]
13.	Tri-D1b-1	0.071[a]	38[b]
14.	Tri-D1b-2	0.060[b]	38[b]
15.	Tri-D1b-3	0.065[b]	36 [b]
16.	Tri-D1b-4	0.072[a]	38[b]
17.	Tri-D1b-5	0.074[a]	36 [b]
18.	Tri-D1b-6	0.063[b]	36 [b]
19.	Tri-A1a-1	0.044[b]	45[a]
20.	Tri-A1a-2	0.040[b]	44[a]
21.	Tri-A1a-3	0.041[b]	44[a]
22.	Tri-A1a-4	0.038[b]	45[a]
23.	Tri-A1a-5	0.038[b]	44[a]
24.	Tri-A1a-6	0.037[b]	44[a]
25.	Tri-A1a-7	0.039[b]	44 [a]
26.	Tri-A1a-8	0.046[b]	45 [a]
27.	Tri-A1b-1	0.023[c]	35[b]
28.	Tri-A1b-2	0.019[c]	34[b]
29.	Tri-A1b-3	0.023[c]	35[b]
30.	Tri-A1b-4	0.019[c]	35[b]
31.	Tri-A1b-5	0.026[c]	35[b]
	SD (±)	0.025	3
	SEM (±)	0.0047	0.676

Values in the same column followed by different superscript letter are significantly different at cutoff *P* value of 5 %

Fig. 7 Comparison of representative bread loaves baked from flour of, *a* NIL Tri-D1a, *b* Recipient variety HD2329, *c* NIL Glu-D1d (subunit 5 + 10), showing comparable effects of triticin and HMW subunits

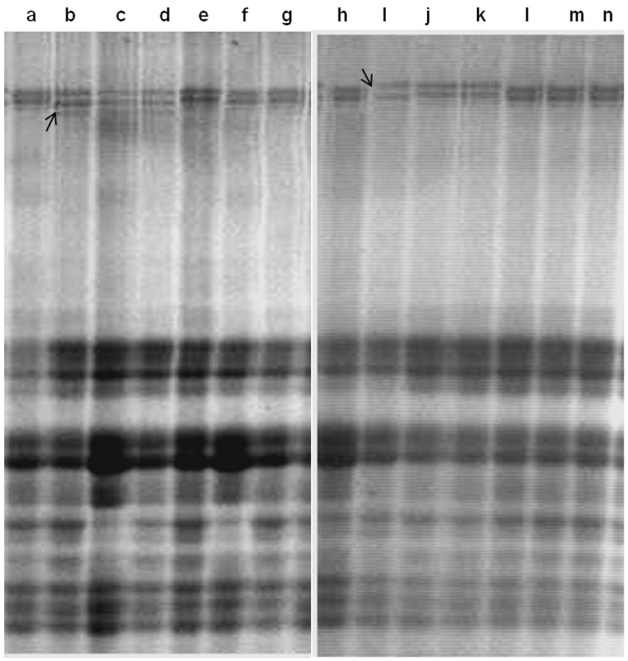

Fig. 8 SDS-PAGE patterns of homozygous F_3 lines selected from segregating bi-parental populations obtained by crossing recipient parent HD2329 with triticin NIL Tri-A1a (*b–g*) and Tri-D1a (*i–n*). *a, h* Recipient parent HD2329, *b–d* lines with triticin allele Tri-A1a (marked with *arrow*), *e–g* lines with triticin allele Tri-A1b (identical to HD2329), *i–k* lines with triticin allele Tri-D1a (marked with *arrow*), *l–n* lines with triticin allele Tri-D1b (identical to HD2329)

also similar behaviour of HMW-GS alleles was observed. Among the alleles present in the different loci of HMW-GS, 1 and 2* at *Glu-A1* locus, 17 + 18 and 7 + 8 at *Glu-B1*, 5 + 10 at *Glu-D1*, *d* at *Glu-A3*, and *b* at *Glu-B3* have all been described as having a positive effect on bread-making quality [4]. Here also we have observed similar behaviour where all the HMW-GS alleles are behaving positively except *Glu-A1c* which is a null allele and behaving negatively. Uthayakumaran et al. also observed that the presence of *Glu-D1d* (subunits 5 + 10) makes a significantly larger contribution to dough properties than those encoded by *Glu-B1* (17 + 18), while subunit 1 encoded by *Glu-A1* made the smallest contribution to functionality [39]. Sontag-Strohm et al. found that progeny carrying allele *Glu-A1b* (subunit 2*) had significantly greater SDS sedimentation-volumes than the null allele *Glu-A1c*, and that adding a HMW glutenin subunit affected extensiograph dough strength more than adding a LMW glutenin subunit, although both increased the SDS-sedimentation volumes [37].

LMW glutenin subunits have also been shown to significantly impact the dough strength in bread wheat [6]. In our study, seven NILs with different LMW glutenin subunits were analysed, and it was found that in comparison to HD2329 allele (*Glu-A3b*) other *Glu-A3* alleles showed negative or no significant effect on bread-making quality, except allele *Glu-A3d* which had a significant positive effect. Earlier Gupta et al. also found association of gliadins and linked *Glu-A3b* allele with dough resistance and extensibility in bread wheat [6]. *Glu-A3a* was found to be superior to *Glu-A3e* and *Glu-A3b* was superior to *Glu-A3c* [5, 7, 8, 17]. In our study, *Glu-A3a, Glu-A3c* and *Glu-A3e* negatively affected the quality, whereas *Glu-A3d, Glu-B3ks* and *Glu-D5a* showed positive effect on the dough properties. Effect of gliadins in determining bread-making quality was variable. Some alleles of γ-gliadins were found to have no significant effect on dough properties, while others were found to be negatively correlated with loaf volume, but none of the Gliadin NILs showed positive effect. Studies on the effect of albumin on wheat quality are limited. Here, we found that four NILs with different alleles of albumin have no significant effect on wheat quality. While effect of glutenin subunits have been reported in several earlier studied, this is the first report on effect of triticin on bread- and chapati-making quality of wheat which can be utilized for the improvement of wheat end-use quality.

Conclusions and Prospects

Significant differences in dough rheological properties and end-use quality for bread and chapati making were observed among NILs with different seed protein alleles in a common genetic background of wheat variety HD2329. While results with the major classes of seed storage proteins namely glutenin and gliadin were confirmatory in nature, a positive effect of wheat storage globulin triticin on the bread- and chapati-making qualities of wheat is demonstrated here for the first time. Also, it is for the first time that the effect of allelic differences at five different classes of seed protein loci have has been analysed in common genetic background. In future, we can study interaction effects of these genes in the common background of HD2329 by making intercrosses and selecting different assortments of alleles. We also need to develop more NILs for a comprehensive coverage of seed storage protein alleles. The information generated here will be very useful in developing high yielding wheat varieties with improved end-use quality.

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